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Work has been done on developing both mononuclear and dinuclear metal complexes for hydrolyzing phosphate esters. What is learned from the study of mononuclear metal complexes can be used to develop more reactive dinuclear metal complexes.

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"Rapid Cleavage of RNA with a La(III) Dimer"
J. Am. Chem. Soc. (1996) p 9982.

Jin Seog Seo, Nack-Do Sung, Rosemary C. Hynes and Jik Chin
"Structure and Reactivity of a Dinuclear Co(III) Complex with a Bridging Phosphate Monoester"
Inorg. Chem. (1996) p 7472.

Jik Chin, Bryan K. Takasaki
"La(III)-Hydrogen Peroxide Cooperativity in Phosphate Diester Cleavage: A Mechanistic Study"
J. Am. Chem. Soc. (1995) p 8582.

Jik Chin, Mary Jane Young
"Dinuclear Copper(II) Complex That Hydrolyzes RNA"
J. Am. Chem. Soc. (1995) p 10577.

Jik Chin, Daphne Wahnon, Anne-Marie Lebuis
"Hydrolysis of a Phosphate Diester Doubly Coordinated to a Dinuclear Cobalt(III) Complex: A Novel Mechanism"
Angew. Chem. Int. Ed. (1995) p 2412.

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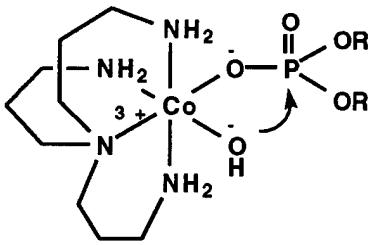
FINAL REPORT

We have now completed our second three year US Army contract (1994 to 1997). During our first three year contract (1990 to 1993) our main focus was on developing simple mononuclear metal complexes that hydrolyze a variety of unactivated substrates by a unified mechanism (Our first US Army contract (1990 to 1993)). This approach led to the development of some of the most reactive mononuclear metal complexes known for hydrolyzing esters, amides, nitriles and phosphate esters. Much of these results were summarized in a review article.[1]

In the second three year contract, we have been working on developing both mononuclear and dinuclear metal complexes for hydrolyzing phosphate esters. As shown below, what is learned from the study of mononuclear metal complexes can be used to develop more reactive dinuclear metal complexes.

Mononuclear metal complexes

During our first three year contract, we showed that the reactivity of *cis*-diaqua Co(III) complexes for hydrolyzing phosphate esters is highly sensitive to the the tetraamine ligand structure.[2,3] For example we showed that the phosphate diester bond in **1** is hydrolyzed about 300 times more rapidly than that in **2** because the O-Co-O bond in **1** is smaller than that in **2**. The value of the O-Co-O bond angle can be decreased by increasing the N-Co-N bond angle directly opposite the O-Co-O bond angle. The N-Co-N bond angle in **1** is larger than that in **2** since the two nitrogens in **1** are connected by three carbon atoms while those in **2** are connected by two carbon atoms.



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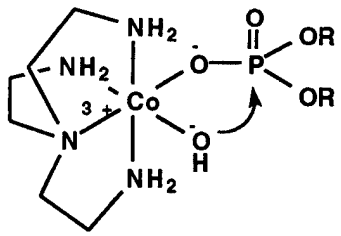
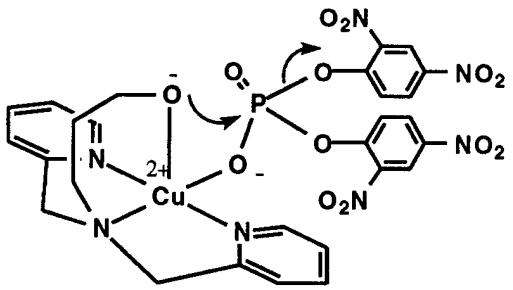


2

We have extended the above concept to include transesterification reactions.[4] Thus, the phosphate diester bond in **3** is cleaved much more rapidly than that in **4** by transesterification. The O-Cu-O bond angle in **3** is expected to be smaller than that in **4** since the O-Cu-N (sp3) bond angle in **3** is greater than that in **4**.



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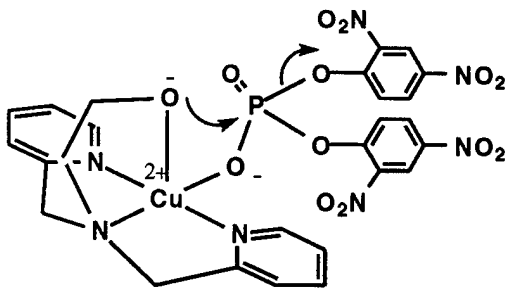
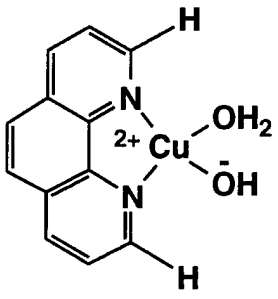


4

In another study, we found that the relative reactivity of **5**, **6** and **7** for hydrolyzing 2',3'-cAMP is amazingly widespread (1 to 10^2 to 10^5). The greater reactivity of **6** over **5** can be explained in terms of the smaller O-Cu-O bond angle in **6** (due to steric hindrance by the methyl groups).[5]



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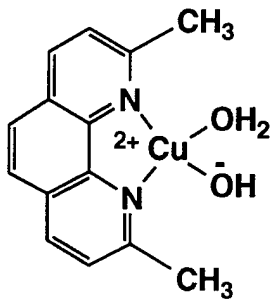

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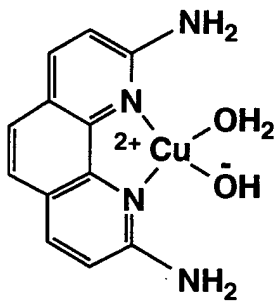
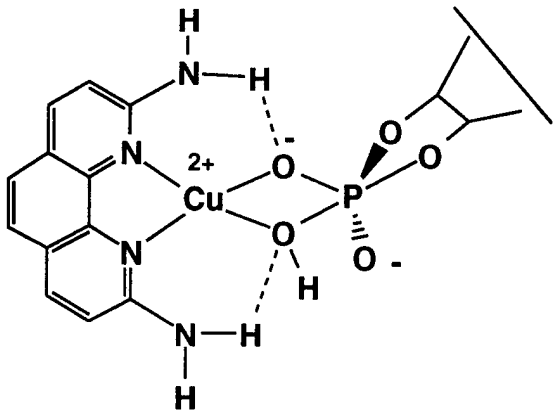


7

It is quite astonishing that **7** is about five orders of magnitude more reactive than **5** for hydrolyzing 2',3'-cAMP.[6] The enormous reactivity of **7** can be explained in terms of hydrogen bond donation by the amino groups to the metal bound water molecules which makes the metal-bound water molecules more acidic. The hydrogen bond also makes it easier to deprotonate the OH group in **8** which in turn facilitates the expulsion of the poor leaving group.



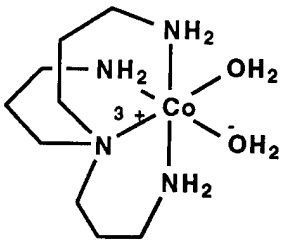
8

Compound **6** has been shown to be highly reactive for cleaving RNA by chelating the phosphate diester bond.[5] We therefore systematically investigated factors affecting chelation of carboxylates and phosphates to metal centers.[7]

Dinuclear Metal Complexes

In nature there are many enzymes that hydrolyze phosphate monoesters, diesters and triesters that are activated by two or more metal ions.[8] Dinuclear aminopeptidases have also been shown to be efficient catalysts for hydrolyzing phosphate triesters.[9] Crystallographic studies revealed that the intermetal distances in many of the above enzymes range from 3 to 5 Å. Over the last few years we have been interested in developing a unified mechanistic approach to developing simple dinuclear metal complexes that hydrolyze phosphate mono-, di- and triesters with good or poor leaving groups. It is important to study the hydrolyses of all types of phosphate esters since the reactions are all mechanistically interrelated. Within this class of reactions, understanding one reaction will help in the understanding of other reactions. Inspired by nature, we designed our dinuclear metal complexes with intermetal distances that range from 3 to 5 Å.

Initially we studied the interations of two mononuclear metal complexes for hydrolyzing phosphate mono[10] and diesters.[11] While addition of one equivalent of **9** to phosphate monoesters with poor leaving groups results in the formation of a stable complex, addition of a second equivalent of **9** results in rapid hydrolysis of the monoester. In another study, we showed that the rate of cleavage of an RNA model compound (**HPNP**) is second order in the concentration of **10**.



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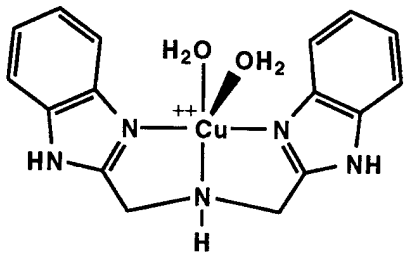

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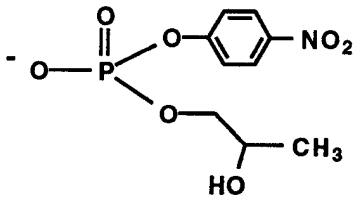
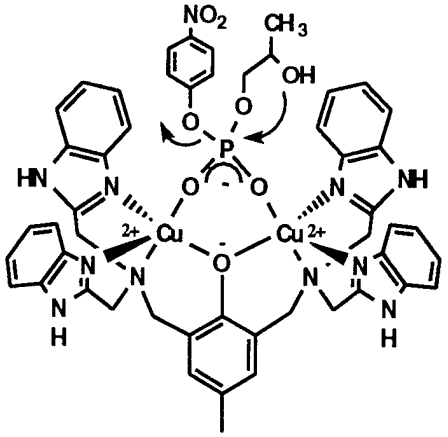


HPNP

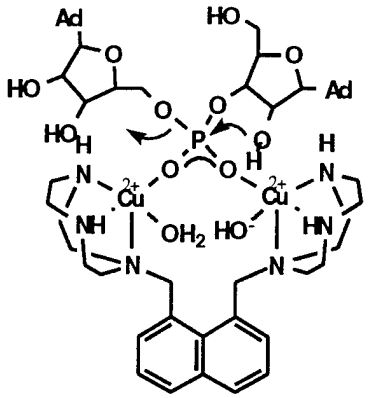
Encouraged by the above results, we developed a dinuclear Cu(II) complex (**11**) that efficiently hydrolyze **HPNP**.[12] This dinuclear complex was found to be much more reactive than any previously reported mononuclear metal complexes for hydrolyzing **HPNP**. Furthermore, crystallographic studies revealed that phosphate diesters can bridge the two metal centers and the intermetal distance in the phosphate diester bridged complex

is about 3.7 Å. This was the first indication that double Lewis acid activation can provide substantial rate accelerations for cleaving phosphate diesters.



11

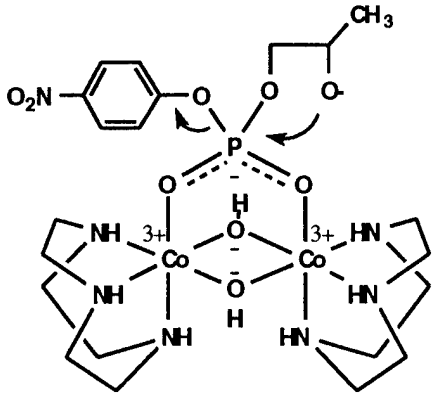
Although **11** is active for hydrolyzing **HPNP**, it turned out not to be reactive for hydrolyzing RNA. We suspected that the lack of reactivity may be due to the bulky ligands in **11** and therefore developed a less bulky dinuclear Cu(II) complex (**12**).[13] This dinuclear complex (**12**) is much more reactive than the corresponding mononuclear metal complex for hydrolyzing RNA. Crystallographic studies showed that phosphate diesters can bridge the two metal centers in **12** and the intermetal distance in the phosphate diester bridged complex is about 5.0 Å.



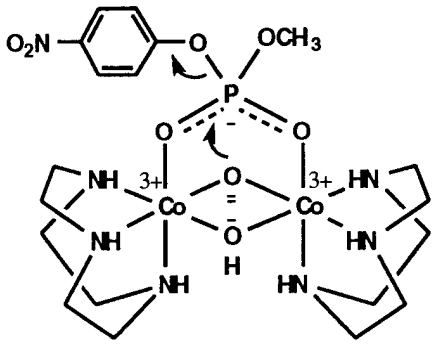
12

In order to quantitate the effect of bridging a phosphate diester over two metal centers (**11** & **12**) we studied the reactivity of subsititutionally innert dinuclear Co(III) complexes with bridging phosphatediesters. We showed that double Lewis acid activation alone provides about 10^6 fold rate acceleration for cleaving phosphate diesters (**13a**).[14] Combination of double Lewis acid activation and bridging oxide activation provides up to 12 orders of magnitude rate acceleration for hydrolyzing phosphate diesters (**13b**).[15]

Crystallographic studies showed that the intermetal distance in **13a** or **13b** is about 3 Å. Interestingly, the structure of the dinuclear metal complex is remarkably similar to that of the active site of purple acid phosphatases and aminopeptidases. The dinuclear Co(III) complex also provides unprecedented rate accelerations for hydrolyzing phosphate monoesters.[16]

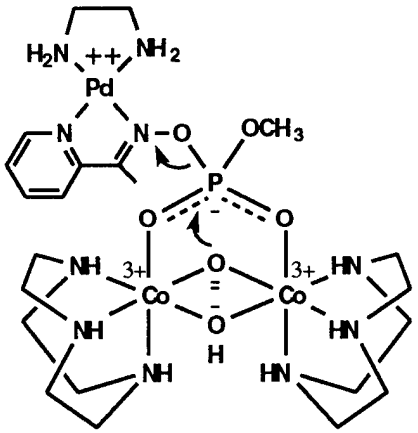


13a

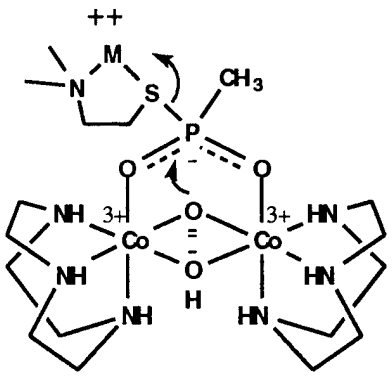


13b

We are interested in using the above mentioned dinuclear Co(III) complex to understand the hydrolysis of not only phosphates with good leaving groups but also those with poor leaving groups. Unfortunately, phosphate diesters with poor leaving groups dissociate from the dinuclear complex before any observable hydrolysis of the diester. However, coordination of a third metal to the leaving group can provide enormous rate acceleration for the hydrolysis reaction. We find that the phosphate diester in **14a** hydrolyzes about 10^{17} times more rapidly than the corresponding metal free phosphate diester.[17] Hydroxide catalyzed hydrolysis of VX results in formation of a thiophosphate diester analog that is as toxic as VX itself. The trinuclear approach shown below (**14b**) may be useful for hydrolyzing the thiophosphate diester analog.

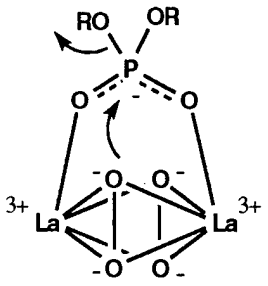


14a



14b

Co(III) complexes are ideal for studying reaction mechanisms in detail because they are substitutionally innert. However they make poor catalysts for the same reason. What is learned from Co(III) complexes can be applied to substitutionally labile metal complexes for developing true catalysts. We have shown that substitutionally labile dinuclear Ln(III) complexes with bridging peroxides[18,19] or hydroxides[20] are highly reactive for hydrolyzing phosphate diesters with good leaving groups as well as those with poor leaving groups including RNA[20] and DNA.[21] Based on O-18 labelling experiments[19] we proposed that the mechanism for the dinuclear Ln(III) complex promoted hydrolysis of activated phosphate diesters involves nucleophilic attack of the bridging peroxide on the bridging phosphate diester (**15**) much like in the dinuclear Co(III) complex where the bridging oxide is the nucleophilic catalyst for hydrolyzing the bridging phosphate diester (**13b**).[15]



15

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Rapid Hydrolysis of 2',3'-cAMP with a Cu(II) Complex: Dramatic Effect of Intramolecular Hydrogen Bonding on Acidity and Reactivity

There is much current interest in developing catalysts that efficiently hydrolyze phosphate diesters with good[1] (NAD, 5'-Capped RNA, ATP) or poor leaving groups[2] (RNA, DNA, phospholipid). It is important to study the hydrolysis of phosphate diesters with both good and poor leaving groups since they may hydrolyze by different mechanisms. In general catalysts that are reactive for hydrolyzing activated substrates may not be reactive for hydrolyzing unactivated ones.[3] Conversely, metal complexes that are not reactive for hydrolyzing phosphates with good leaving groups may nevertheless be active for hydrolyzing phosphates with poor leaving groups.[4] Here we report a simple Cu(II) complex (**1**) that is highly reactive for hydrolyzing phosphate diesters with good (bis(2,4-dinitrophenyl)-phosphate: BDNPP) or poor leaving groups (2',3'-cAMP).

(Structures **1**, **2**, and **3**)

2,9-Diamino-*o*-phenanthroline was synthesized according to a literature procedure.[5] [(2,9-Diamino-*o*-phenanthroline)$CuCl_2$] was prepared from a methanolic solution of $CuCl_2$ and 2,9-Diamino-*o*-phenanthroline. Copper complexes **1**, **2** and **3** were freshly generated in water from the corresponding chlorides. Cleavage of ApA and 2',3'-cAMP was monitored by HPLC as described previously[6] and cleavage of BDNPP was monitored by UV/VIS methods.[7] The reaction solutions were buffered by the metal complexes.[8] A typical HPLC plot for **1** promoted cleavage of 2',3'-cAMP (0.05 mM) at pH 5 and 25 °C is shown in Figure 1. It is evident from the HPLC plot that the cleavage reaction occurs hydrolytically producing 3'-AMP and 2'-AMP in a ratio of about 16 to 1.

In general it is difficult to study the hydrolysis of phosphate diesters with poor leaving groups under mild conditions because of their enormous stability. 2',3'-cAMP is an ideal compound for such studies since despite the poor leaving groups it hydrolyzes almost as rapidly as BDNPP under basic conditions due in large part to the five-membered ring strain (Table I). It has been reported that **2** and **3** are reactive for hydrolyzing 2',3'-cAMP but that **3** is not reactive for hydrolyzing BNPP (bis(p-nitrophenyl)phosphate).[4] We find that **1** is much more reactive than **2** or **3** for hydrolyzing BDNPP and 2',3'-cAMP.

Potentiometric titration reveals that the pK_a values of the protonated metal-hydroxides in **1**, **2** and **3** are 5.5, 7.0 and 8.2 respectively. It is surprising that the amino

group in **1** lowers the pK_a of the metal bound water molecule given that it is an electron donating group which should lower the positive charge on the metal and increase the pK_a of the metal bound water. It appears that the amino group is acting as a hydrogen bond donor to the metal bound water molecule thereby lowering the pK_a (**1H**). The delocalization of the lone pair electrons on the amino nitrogen should direct the positioning of the hydrogen bond. As shown below this intramolecular hydrogen bonding may have dramatic consequences for the the reactivity of the metal complex for cleaving phosphate diesters.

(Structure **1H**)

The rates of hydrolysis of BDNPP and 2',3'-cAMP with each of the three metal complexes increase with increase in the solution pH but level off above the respective pK_as of the coordinated water molecules. Hence it is the hydroxy forms of the metal complexes that are active for hydrolyzing the phosphate diesters. Figure 2 shows the pH-rate profiles for **1** promoted hydrolysis of BDNPP and 2',3'-cAMP.[9] The reactivities of the free hyroxide and the three metal-hydroxide complexes for hydrolyzing BDNPP and 2',3'-cAMP are listed in Table I.

Complex **1** is over four orders of magnitude more reactive than **3** for hydrolyzing BDNPP as well as for hydrolyzing 2',3'-cAMP (Table I). Furthermore, **1** is more reactive than free hydroxide for hydrolyzing BDNPP[10] and 2',3'-cAMP[11] by over three and four orders of magnitude respectively. At pH 6, **1** (1 mM) provides over a billion fold rate-acceleration for the hydrolysis of 2',3'-cAMP over the background hydroxide rate.[12]

It is interesting to compare the reactivities of **1** and **2** for cleaving various phosphate diesters. Amazingly, complex **1** is almost three orders of magnitude more reactive than **2** for hydrolyzing 2',3'-cAMP yet the second order rate constant for **1** promoted cleavage of ApA at pH 5.5 and 25 °C (2.8×10^{-1} M^{-1} s^{-1}) is comparable to that for **2** promoted cleavage of ApA at pH 7.0 and 25 °C (3.9×10^{-1} M^{-1} s^{-1}).[6] What then could be the role of the amino group? Since both phosphate diester have poor leaving groups it is unlikely that the amino group is providing general acid catalysis for the expulsion of the leaving group in 2',3'-cAMP but not for that in ApA. It is also unlikely that the amino group is acting as a nucleophilic catalyst for hydrolyzing 2',3'-cAMP since **4** does not hydrolyze to any appreciable extent under the same experimental conditions used to hydrolyze 2',3'-cAMP.

(Structure **4**)

We propose that the mechanism for **1** and **2** promoted hydrolysis of BDNPP and 2',3'-cAMP involves intramolecular nucleophilic attack by the metal-hydroxide on the coordinated phosphate diester Such a mechanism has been proposed for *cis*-diaqua Co(III) complex promoted hydrolysis of phosphate diesters with good or poor leaving groups.[7,13] The reason why **1** is more reactive than **2** for hydrolyzing 2',3'c-AMP may be that the amino group assists in deprotonation of the OH group in **5** by acidifying it (as in **1H**). This deprotonation should facilitate the expulsion of the leaving group. The extent of this deprotonation at the transition state is expected to be greater for phosphate diesters with poorer leaving group.[14] Hence the difference in the reactivity of **1** and **2** for hydrolyzing BDNPP is only about 20 fold while that for 2',3'-cAMP is about 600 fold. To be consistent with the observed pH-rate profile, water should be the base that deprotonates this OH group. In order to test the hypothesis that the amino group in **1** assists in deprotonation of the OH group in **5**, we compared the rates of **1** promoted hydrolysis (**5**) and methanolysis (**6**) of 2',3'-cAMP. No methanolysis could be observed under the same condition used to hydrolyze 2',3'-cAMP. In contrast, the rates of **1** promoted methanolysis and hydrolysis of BDNPP are comparable. It appearst that deprotonation of the OH group in **5** is important for hydrolyzing phosphates with poor leaving groups but not those with good leaving groups. It is unlikely that the amino group is acting as a general base catalyst to directly deprotonate the OH group in **5** since its basicity is expected to be too weak. For example, the pK_a value of protonated 2-aminopyridinium is only -7.6.[15]

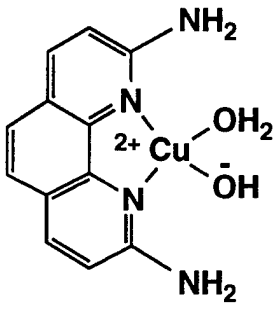
(Structures **5** and **6**)

Although the metal-hydroxides in **1** and **2** may act as highly efficient intramolecular nucleophilic catalysts for hydrolyzing BDNPP and 2',3'-cAMP, they cannot compete with the 2'-hydroxyl group of RNA which is known to provide over nine orders of magnitude rate-acceleration for cleaving the phosphate diester.[16] We proposed that the mechanism for **2** promoted cleavage of ApA involves chelation of the phosphate diester followed by intramolecular nucleophilic attack by the 2'-hydroxyl group.[6] It is likely that **1** and **2** are cleaving ApA by the same mechanism since the structures and the reactivities of the two metal complexes are similar. In this mechanism the role of the amino groups in **1** is apparently no different from that of the methyl groups in **2**.

Interestingly, **1** is not only the most reactive for hydrolyzing 2',3'-cAMP but also the most regiospecific. For hydroxide and **3** promoted hydrolysis of 2',3'-cAMP, the ratios of the two adenosine monophosphate products (3'-AMP/2'-AMP) are about one whereas

those for **1** and **2** are 16 and 6 respectively. The exact origin of this regiospecificity is unclear although both hydrogen bonding and hydrophobic interaction may be important.

In conclusion, **1** provides over a billion fold rate acceleration over the background hydroxide rate (1 mM metal complex at pH 6, 25 °C) for hydrolyzing 2',3'-cAMP by a novel mechanism involving intramolecular hydrogen bonding. This represents by far the most reactive and also the most regiospecific transition metal complex reported to date for hydrolyzing 2',3'-cAMP.[17]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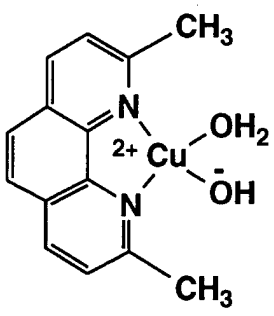
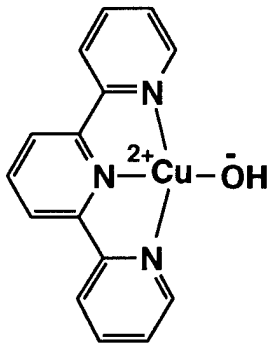
N
2+
Cu
OH2
OH
N
NH2

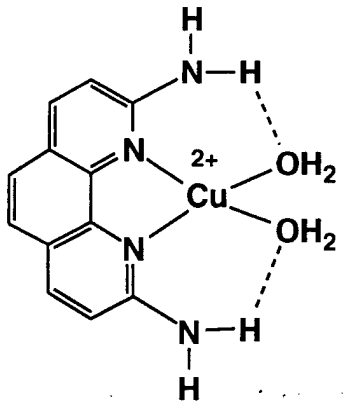
1


CH3
N
2+
Cu
OH2
OH
N
CH3

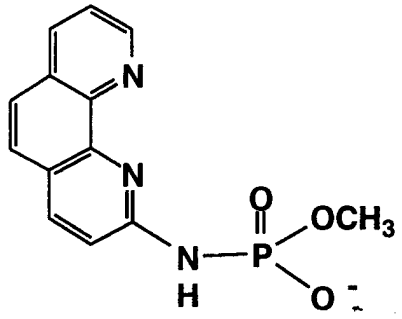
2


N
2+
N
Cu
OH
N

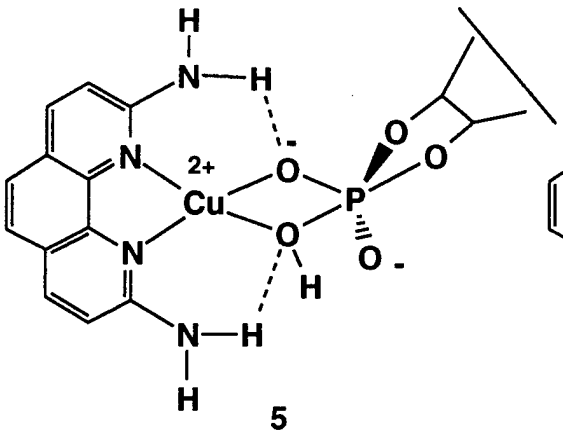
3


H
N
H
N
2+
Cu
OH2
OH2
N
N
H
H

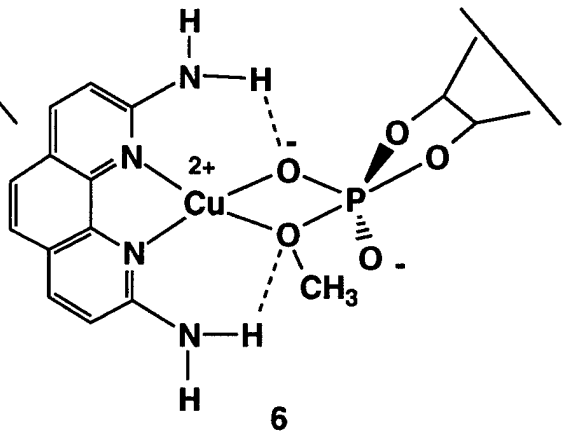
1H


N
N
O
OCH3
N
P
H
O

4


H
N
H
N
2+
Cu
O
P
O
O
O
H
N
H
H

5


H
N
H
N
2+
Cu
O
P
O
O
O
CH3
N
H
H

6

Table I. Second order rate constants (M^{-1} s^{-1}) for hydrolysis of phosphate diesters at 25 °C.

	BDNPP (k)	BDNPP (rel)	2',3'-cAMP (k)	2',3'-cAMP (rel)
OH	3.2×10^{-3}	1	1.1×10^{-3}	1
3	5.7×10^{-4}	1.8×10^{-1}	5.1×10^{-3}	4.6×10^{0}
2	8.0×10^{-1}	2.5×10^{2}	6.2×10^{-2}	5.6×10^{1}
1	2.0×10^{1}	6.3×10^{3}	3.8×10^{1}	3.5×10^{4}

Figure 1. HPLC traces of **1** (1 mM) promoted cleavage of 2',3'-cAMP (0.05 mM) at 25 °C, pH 5.0. Retention times are as follows: 3'-AMP = 3.5 min; 2',3'-cAMP = 7.5 min; 2'-AMP = 8 min. Reaction times are from foreground to background, 6, 30, 120, 306, and 630 s.

Figure 2. pH-rate profiles for **1** (1 mM) promoted hydrolysis of BDNPP (10^{-5} M) and 2',3'-cAMP (0.05 mM) at 25 °C.

Abstract

The Copper (II) complex of 2,9-diamino-*o*-phenanthroline (**1**) hydrolyzes 2',3'-cAMP to give 3'-AMP and 2'-AMP in a ratio of 16 to 1. It provides over a billion fold rate acceleration over the background hydroxide rate ($k = 3.8 \times 10^{-2}$ s-1 at 1 mM metal complex, pH 6, 25 °C) and it is about six hundred times more reactive than the Copper (II) complex of 2,9-dimethyl-*o*-phenanthroline for hydrolyzing 2',3'-cAMP . The reactivity of the Cu(II) complex (**1**) can be explained in terms of intramolelcular hydrogen bonding between the amino groups and the metal bound water molecules.

---

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8 We were not able to find a buffer system that does not decrease the activity of **1** for cleaving ApA. The pH of the reaction solution did not change by more than 0.1 during the cleavage reaction.

9 The pH-rate profiles were fit according to $k_{obs} = kK_a[\mathbf{M}]/([H] + K_a)$ (where [**M**] represents the concentrations of the metal complex, K_a is the acid dissociation constant of the metal bound water and k is the second order rate constant for **1** promoted hydrolysis of BDNPP or 2',3'-cAMP). All of the reactions were followed to at least three half-lives and the rates were reproducible to within 3% error.

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12 This is not the experimentally observed rate-acceleration at pH 6 since the water rate for 2',3'-cAMP hydrolysis is not known. However the water rate may be insignificant down to pH 6 as in ApA cleavage. Järvinen, P.; Oivanen, M.; Lönnberg, H. *J. Org. Chem.* **1991**, *56*, 5396.

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14 Extent of bond making and breaking in transition state **5** is not indicated. This reaction may well be concerted (Hengge, A. C.; Cleland, W. W. *J. Am. Chem. Soc.* **1990**, *112*, 7421) since the metal-hydroxide is expected to be as good a leaving group as p-nitrophenol.

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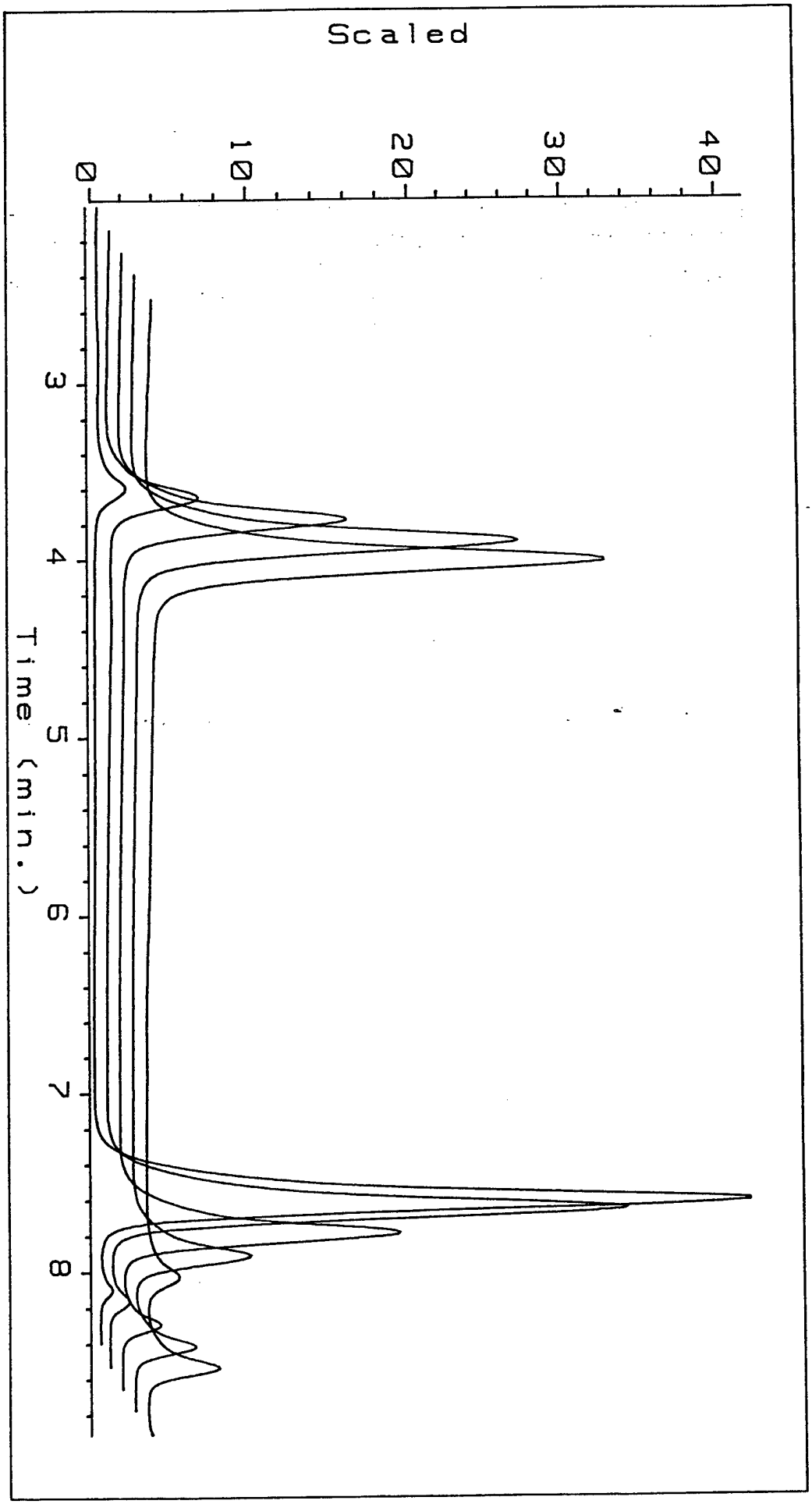
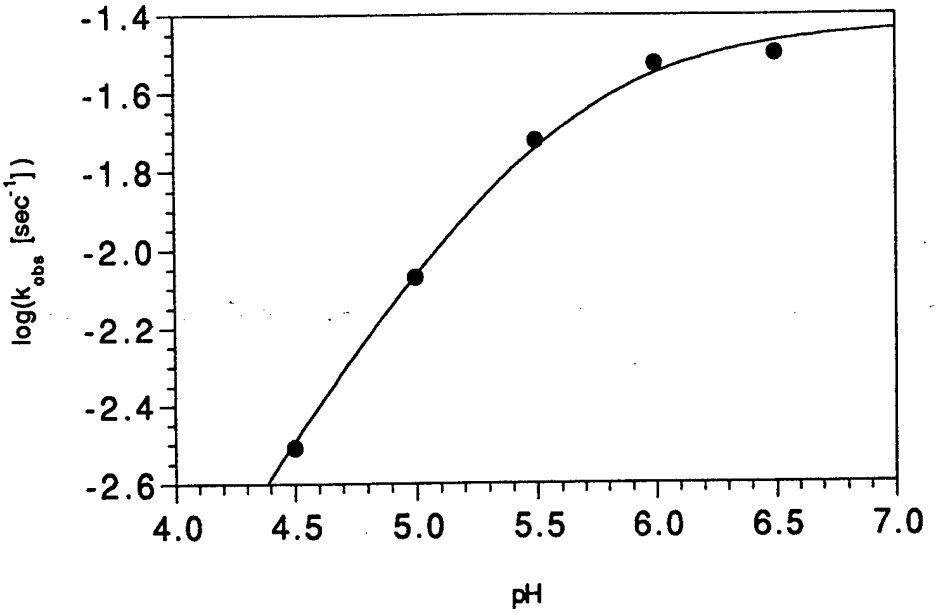
17 Ligand free Ce(IV) is more reactive than **1**. See for example (a) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1994**, *116*, 1121. (b) Sumaoka, J.; Miyama, S.; Komiyama, M. *J. Chem. Soc. Chem. Commun.*, **1994**, 1755.


Scaled
0
10
20
30
40
3
4
5
6
7
8
Time (min.)


-1.4
-1.6
-1.8
-2.0
-2.2
-2.4
-2.6
log(k_obs [sec^-1])
4.0
4.5
5.0
5.5
6.0
6.5
7.0
pH